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## **Halobacterium bonnevilliei sp. Nov., halobaculum saliterrae sp. nov. and halovenus carboxidivorans sp. nov., three novel carbon monoxide-oxidizing halobacteria from saline crusts and soils**

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# *Halobacterium bonnevilliei* sp. nov., *Halobaculum saliterrae* sp. nov. and *Halovenus carboxidivorans* sp. nov., three novel carbon monoxide-oxidizing Halobacteria from saline crusts and soils

Marisa R. Myers† and G.M. King\*,†

## Abstract

Three novel carbon monoxide-oxidizing Halobacteria were isolated from Bonneville Salt Flats (Utah, USA) salt crusts and nearby saline soils. Phylogenetic analysis of 16S rRNA gene sequences revealed that strains PCN9<sup>T</sup>, WSA2<sup>T</sup> and WSH3<sup>T</sup> belong to the genera *Halobacterium*, *Halobaculum* and *Halovenus*, respectively. Strains PCN9<sup>T</sup>, WSA2<sup>T</sup> and WSH3<sup>T</sup> grew optimally at 40 °C (PCN9<sup>T</sup>) or 50 °C (WSA2<sup>T</sup>, WSH3<sup>T</sup>). NaCl optima were 3 M (PCN9<sup>T</sup>, WSA2<sup>T</sup>) or 4 M NaCl (WSH3<sup>T</sup>). Carbon monoxide was oxidized by all isolates, each of which contained a molybdenum-dependent CO dehydrogenase. G+C contents for the three respective isolates were 66.75, 67.62, and 63.97 mol% as derived from genome analyses. The closest phylogenetic relatives for PCN9<sup>T</sup>, WSA2<sup>T</sup> and WSH3<sup>T</sup> were *Halobacterium noricense* A1<sup>T</sup>, *Halobaculum roseum* D90<sup>T</sup> and *Halovenus aranensis* EB27<sup>T</sup> with 98.71, 98.19 and 95.95% 16S rRNA gene sequence similarities, respectively. Genome comparisons of PCN9<sup>T</sup> with *Halobacterium noricense* A1<sup>T</sup> yielded an average nucleotide identity (ANI) of 82.0% and a digital DNA–DNA hybridization (dDDH) value of 25.7%; comparisons of WSA2<sup>T</sup> with *Halobaculum roseum* D90<sup>T</sup> yielded ANI and dDDH values of 86.34 and 31.1%, respectively. The ANI value for a comparison of WSH3<sup>T</sup> with *Halovenus aranensis* EB27<sup>T</sup> was 75.2%. Physiological, biochemical, genetic and genomic characteristics of PCN9<sup>T</sup>, WSA2<sup>T</sup> and WSH3<sup>T</sup> differentiated them from their closest phylogenetic neighbours and indicated that they represent novel species for which the names *Halobaculum bonnevilliei*, *Halobaculum saliterrae* and *Halovenus carboxidivorans* are proposed, respectively. The type strains are PCN9<sup>T</sup> (=JCM 32472=LMG 31022=ATCC TSD-126), WSA2<sup>T</sup> (=JCM 32473=ATCC TSD-127) and WSH3<sup>T</sup> (=JCM 32474=ATCC TSD-128).

Extremely halophilic archaea, phylum Euryarchaeota, class *Halobacteria*, occur ubiquitously in anthropogenic and natural saline and hypersaline environments [1–3]. At the time of writing, at least 65 genera have been validly published, with the overwhelming majority of halobacteria described as aerobic or facultatively anaerobic heterotrophs. Genomic and cultivation-based analyses have shown that many of these halobacteria harbour genes for unexpected traits, e.g., the ability to oxidize carbon monoxide (CO) using the form I molybdenum-dependent CO dehydrogenase (Mo-CODH) [4]. Globally, approximately 10% of annual CO emissions are consumed by microbial species in soils, with the capacity

haloarchaeal isolates to oxidize CO only recently discovered [5–7]. Several isolates obtained from the Bonneville Salt Flats (BSFs; Utah, USA) that contain these genes have been shown to couple CO oxidation to perchlorate reduction in addition to molecular oxygen and nitrate reduction [8, 9].

The BSFs, located in the western part of the Great Salt Lake Desert, is a large playa known for its salt crust [10–12]. A remnant of Pleistocene Lake Bonneville, it supports a predominantly halite crust, which undergoes partial dissolution during winter and recrystallization during summer. We report here the isolation of three CO-oxidizing haloarchaeal species obtained during an investigation of BSF and

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**Keywords:** haloarchaea; carbon monoxide; bonneville salt flats.

**Abbreviations:** ANI, average nucleotide identity; BSF, Bonneville Salt Flats; CO, carbon monoxide; *coxL*, large sub-unit gene for CO dehydrogenase; dDDH, digital DNA–DNA hybridization; DSMZ, German Collection of Microorganisms and Cell Cultures; GOLD, Genomes OnLine database; IMG, Integrated Microbial Genomes Microbial Annotation Pipeline; JCM, Japan Collection of Microorganisms; Mo-CODH, form I molybdenum-dependent CO dehydrogenase.

The 16S rRNA gene sequences have been deposited as: *Halobacterium bonnevilliei* MK262901. *Halobaculum saliterrae* MF767880. *Halovenus carboxidivorans* MN128599. Genomes have been deposited at DDBJ/ENA/GenBank under the following accessions: *Halobaculum bonnevilliei* WUUU000000000. *Halobaculum saliterrae* WUUS000000000. *Halovenus carboxidivorans* WUUT000000000.

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One supplementary table and three supplementary figures are available with the online version of this article.

neighbouring saline soils. *Halobacterium* PCN9<sup>T</sup> was isolated from a BSF salt crust, while strains *Halobaculum* WSA2<sup>T</sup> and *Halovenus* WSH3<sup>T</sup> were isolated from nearby saline soils. These taxa were recognized as novel extreme halophiles based on their 16S rRNA gene sequences and other distinct traits [13, 14].

Salt crusts and saline soils were collected from the BSFs (40.757333° N, 113.886417° W) and a site south of Wendover, UT (40.515778° N, 114.045056° W) during July 2015. Enrichments were performed by transferring approximately 0.5 g fresh weight of crust or soil samples into 60 ml serum bottles containing 5 ml CM1 medium with 3.8 M NaCl and 25 mM pyruvate [7, 15]; the bottles were then sealed with neoprene rubber stoppers. Bottle headspaces contained either air (oxic conditions) or oxygen-free nitrogen (anoxic conditions) to which CO was added at a final concentration of 100 p.p.m. Potassium nitrate was added at a final concentration of 10 mM to anoxic bottles. Bottles were then incubated at 40 or 60 °C. Headspace CO was monitored at intervals as previously described [7]. Sub-samples from enrichments that oxidized CO were used to inoculate fresh 3.8 M NaCl CM1-pyruvate media; this process was repeated three times prior to spreading serial dilutions onto 3.8 M NaCl CM1-pyruvate agar plates. Plates were incubated at 40 or 55 °C corresponding to the initial enrichment incubation temperature. After approximately 7 days, morphologically distinct colonies were selected and used to inoculate 60 ml serum bottles containing 5 ml 3.8 M NaCl CM1-pyruvate media with air headspaces. Cultures were screened for CO oxidation as before; positive cultures were re-plated onto 3.8 M NaCl CM1-pyruvate plates as needed to obtain pure isolates. Three novel isolates (PCN9<sup>T</sup>, WSA2<sup>T</sup> and WSH3<sup>T</sup>) were selected for detailed characterization.

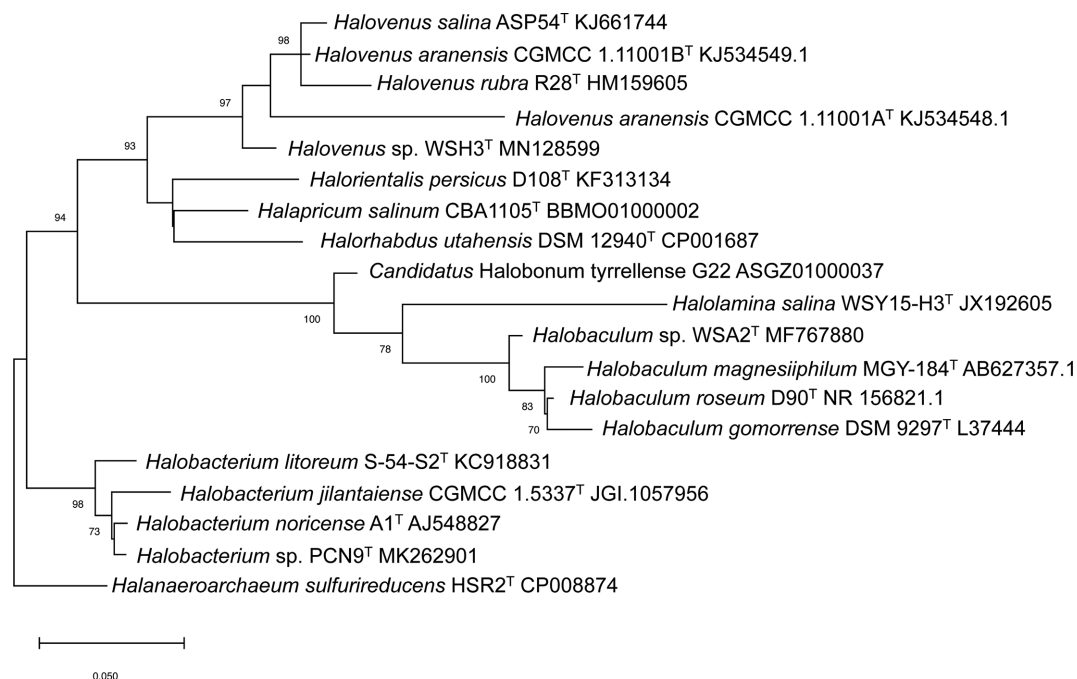
Two additional strains, *Halobaculum roseum* D90<sup>T</sup> and *Halobacterium noricense* A1<sup>T</sup>, that were close phylogenetic neighbours of WSA2<sup>T</sup> and PCN9<sup>T</sup>, respectively, were obtained from the Japan Collection of Microorganisms (JCM) and the German Collection of Microorganisms and Cell Cultures (DSMZ), respectively. Both strains were grown in 3.8 M NaCl CM1-pyruvate. CO uptake potential was assessed by adding 10 p.p.m. CO to 5 ml culture in 60 ml serum bottles containing an aerobic headspace, and monitoring as previously described. Neither isolate D90<sup>T</sup> nor A1<sup>T</sup> oxidized exogenous CO.

Genomic DNA was extracted from strains PCN9<sup>T</sup>, WSA2<sup>T</sup> and WSH3<sup>T</sup> using a MoBio UltraClean DNA extraction kit following the manufacturer's instructions (Folsom). Genomic DNA extracts were used to amplify 16S rRNA genes using archaeal forward primer Arch21F (5'-TTCCG-GTTGATCCYGCCGGA-3' [16, 17]; and universal reverse primer 1492R (5'-CGGTTACCTTGTACGACTT-3' [18]. Amplicons purified with a MoBio UltraClean PCR Clean-up kit were sequenced using an ABI 3130XL Genetic Analyzer (Applied Biosystems) at the Louisiana State University Genomics Facility (Baton Rouge, LA, USA). The resulting bidirectional sequence reads were assembled and edited using Sequencher 4.8 (Gene Codes Corporation). The 16S rRNA

gene sequences have been deposited in Genbank as accessions MK262901, MF767880 and MN128599 for *Halobacterium* sp. PCN9<sup>T</sup>, *Halobaculum* sp. WSA2<sup>T</sup> and *Halovenus* sp. WSH3<sup>T</sup>, respectively. Based on 16S rRNA gene sequence analysis in EZBioCloud [19], *Halobacterium* sp. PCN9<sup>T</sup>, *Halobaculum* sp. WSA2<sup>T</sup> and *Halovenus* sp. WSH3<sup>T</sup> are most closely related to *Halobacterium noricense* A1<sup>T</sup>, *Halobaculum roseum* D90<sup>T</sup> and *Halovenus aranensis* EB27<sup>T</sup> with gene sequence identities of 98.71, 98.19 and 95.95% respectively [20–22]. All sequence identities are at or below the accepted cutoff for 16S rRNA gene-based species differentiation of 98.7–99.1% [23]. Phylogenetic relationships of the novel isolates and close relatives were determined using a maximum likelihood analysis in MEGA X (Fig. 1 [24–26]. Additionally, maximum-parsimony and neighbour-joining analyses were performed which supported the maximum-likelihood analysis (Figs S1 and S2, available in the online version of this article).

The large sub-unit gene for CO dehydrogenase (*coxL*) was amplified using primers ArchcoxF (5'-GGYGGST-TYGGSAASAAGGT-3') and PSr (5'-YTCGAYGATCATCG-GRTTGA-3'). The PCR reaction mixture consisted of 0.2 µl Hotmaster Taq DNA Polymerase (Quantabio), 34.8 µl nuclease-free H<sub>2</sub>O, 5 µl Hotmaster Taq Buffer, 3 µl DMSO, 2 µl dNTPs, 2 µl ArchcoxF primer, 2 µl PSr primer and 1 µl template. Thermocycler conditions included: initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 58 °C for 1 min and elongation at 72 °C for 2 min, followed by a final elongation at 68 °C for 10 min. Amplicons were purified and sequenced as above. *CoxL* sequences have been deposited in Genbank as accessions MN136860, MF773972 and MN136859 for *Halobacterium* sp. PCN9<sup>T</sup>, *Halobaculum* sp. WSA2<sup>T</sup> and *Halovenus* sp. WSH3<sup>T</sup>, respectively.

Genomic analyses for WSA2<sup>T</sup> and WSH3<sup>T</sup> were conducted using paired-end DNA sequences generated on an Illumina Miseq with 2×250 bp chemistry at Michigan State University. Genomes for *Halobacterium noricense* A1<sup>T</sup> and *Halobaculum roseum* D90<sup>T</sup> were sequenced similarly, but with Illumina Miseq and 2×150 bp chemistry. Genomic analysis for PCN9<sup>T</sup> was performed using an Ion Torrent S5 with a 530 chip at Louisiana State University. A total of 8132138, 1402189, 1345632, 1707515 and 1635149 reads were generated for PCN9<sup>T</sup>, WSA2<sup>T</sup>, WSH3<sup>T</sup>, D90<sup>T</sup> and A1<sup>T</sup>, respectively. Reads were assembled using a5\_miseq\_macOS\_20140604 [27] for WSA2<sup>T</sup>, WSH3<sup>T</sup>, D90<sup>T</sup> and A1<sup>T</sup>, and SPAdes-3.10.0-Darwin for PCN9<sup>T</sup> [28]. Assemblies were submitted to the Integrated Microbial Genomes Microbial Annotation Pipeline (IMG) for annotation [29]. Genomes for PCN9<sup>T</sup>, WSA2<sup>T</sup> and WSH3<sup>T</sup> are available within the Genomes OnLine database (GOLD) under study ID Gs0116871 as projects Gp0375023, Gp0252472 and Gp0252468, respectively [30]. Additionally, Whole Genome Shotgun projects have been deposited at DDBJ/ENA/GenBank under the accessions WUUU00000000, WUUS00000000 and WUUT00000000 for PCN9<sup>T</sup>, WSA2<sup>T</sup> and WSH3<sup>T</sup>, respectively. The versions described in this paper are versions WUUU01000000, WUUS01000000 and WUUT01000000.



**Fig. 1.** Evolutionary analysis by the maximum-likelihood method. The evolutionary history was inferred by using the maximum-likelihood method and the general time reversible model. The tree with the highest log likelihood (−4477.23) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying the Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites [five categories (+G, parameter=1.2043)]. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 67.09% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 19 nucleotide sequences. All positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 1108 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.

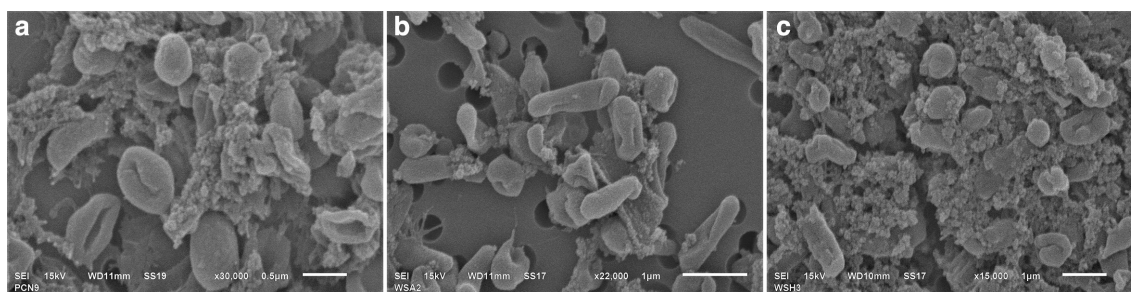
The PCN9<sup>T</sup> genome was composed of 3006633 bp with 85.62% coding DNA and 4003 genes of which 3952 (98.73%) were protein coding. There were 2576 (64.35%) protein-coding genes, 808 of which were associated with KEGG pathways, 1949 with COGs and 620 with MetaCyc pathways. Additionally, one CRISPR, one 16S rRNA gene and 44 tRNA genes were identified. The WSA2<sup>T</sup> genome was composed of 3775766 bp with 87.20% coding DNA and 3800 genes of which 3737 (98.34%) were protein coding, with 855 associated with KEGG pathways, 2062 with COGs and 681 with MetaCyc pathways. Two CRISPR, two distinct 16S rRNA genes and 51 tRNAs were identified. The WSH3<sup>T</sup> genome was composed of 3245916 bp with 90.17% coding DNA and 3337 genes of which 3288 (98.53%) are protein coding with 836 associated with KEGG pathways, 1926 with COGs and 676 with MetaCyc pathways. One 16S rRNA gene and 44 tRNA were identified, but no CRISPR were found. Each of the genomes harboured a *cox* operon comprised of the canonical form I structural genes for the molybdenum-dependent CO dehydrogenase, *coxM*, *coxS*, *coxL*, along with several accessory genes (e.g., [4]).

Genome sequences for D90<sup>T</sup> and A1<sup>T</sup> were used with sequences for WSA2<sup>T</sup> and PCN9<sup>T</sup>, respectively, to conduct digital DNA–DNA hybridization (dDDH) and average nucleotide identity (ANI) analyses using the Genome-to-Genome

Distance Calculator for dDDH (<https://ggdc.dsmz.de/>; [31]) and the EZBcloud ANI calculator ([32]; [www.ezbiocloud.net/tools/ani](http://www.ezbiocloud.net/tools/ani)). Genome comparisons of WSA2<sup>T</sup> with *Halobaculum roseum* D90<sup>T</sup> yielded dDDH and ANI values of 31.1 and 86.34%, respectively; comparisons of PCN9<sup>T</sup> with *Halobacterium noricense* A1<sup>T</sup> yielded dDDH and ANI values of 25.7 and 82.00%, respectively. The ANI value for WSH3<sup>T</sup> and *Halovenus aranensis* EB27<sup>T</sup>, 75.2%, was determined using the Pairwise ANI application of Integrated Microbial Genomes and Microbiomes ([img.jgi.doe.gov](http://img.jgi.doe.gov)). The ANI value for WSH3<sup>T</sup> and dDDH and ANI values for both WSA2<sup>T</sup> and PCN9<sup>T</sup> were consistent with species level differentiation from their closest phylogenetic neighbours.

Cell and colony characteristics were determined after growth for 6 days at 40 °C (PCN9<sup>T</sup> and WSA2<sup>T</sup>) or 55 °C (WSH3<sup>T</sup>) on 3.8 M NaCl CM1-pyruvate medium. For Gram-staining, a modified procedure was used that consisted of a rinse of CM1 containing 1 M NaCl instead of deionized water to prevent cell lysis. All isolates stained Gram-negative. Cell morphology and dimensions were determined using a Zeiss Axioscope and an AxioCam MR digital camera. Cells occurred singly with the following dimensions: PCN9<sup>T</sup>, 2.15±0.31 µm×0.70±0.10 µm; WSA2<sup>T</sup>, 2.33±0.71×0.67±0.15 µm; and WSH3<sup>T</sup>, 2.28±0.18×0.80±0.18 µm. Cells were additionally imaged at





**Fig. 2.** Scanning electron microscopy images of, (a) *Halobacterium bonnevilliei* PCN9<sup>T</sup>; (b) *Halobaculum salitrrae* WSA2<sup>T</sup>; (c) *Halovenus carboxidivorans* WSH3<sup>T</sup>.

LSU's Shared Instrument Facility with a JEOL 6610LV scanning electron microscope. (Fig. 2a–c). For SEM imaging, cells were collected by filtration and fixed on a 0.4 µm polycarbonate filter in 2% glutaraldehyde, 2% formaldehyde, 1% OsO<sub>4</sub> for 30 min. Cells were then rinsed with distilled water for 10 min (×3), dehydrated with a graded ethanol series and dried with a graded HMDS series (hexamethyldisilazane reagent EMS#16700). The filter paper was mounted on aluminum specimen stubs and coated with platinum in an EMS550X Sputter Coater. Both PCN9<sup>T</sup> and WSA2<sup>T</sup> formed round, smooth, convex, shiny, opaque, pink-pigmented colonies, while WSH3<sup>T</sup> formed small round, smooth, convex, shiny, opaque, red-pigmented colonies.

All physiological and biochemical assays were conducted with media based on 3.8 M NaCl CM1-pyruvate, and growth was measured by monitoring absorbance at 600 nm in triplicate unless otherwise noted. Temperature ranges and optima were assessed at intervals from 15–65 °C for 14 days. PCN9<sup>T</sup> grew optimally at 40 °C, while WSA2<sup>T</sup> and WSH3<sup>T</sup> grew optimally at 50 °C with ranges of 15–55 °C (PCN9<sup>T</sup>), 25–60 °C (WSA2<sup>T</sup>) and 30–60 °C (WSH3<sup>T</sup>). The ranges and optimal NaCl concentrations for growth were determined using CM1-pyruvate based media with NaCl concentrations adjusted to 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.4 M; growth was measured for 14 days. PCN9<sup>T</sup> and WSA2<sup>T</sup> grew optimally at 3 M with a range of 2.0–5.4 M, WSH3<sup>T</sup> grew optimally at 4 M with a range of 3.0–5.4 M (Table 1).

The minimum concentration of added magnesium (Mg<sup>2+</sup>) required for growth was assessed using a modified 3.8 M NaCl CM1 medium in which magnesium sulfate was replaced with sodium sulfate, and magnesium chloride was replaced with sodium chloride. Magnesium chloride was then added to final concentrations of 0, 1, 5 and 10 mM; growth was monitored for 14 days. PCN9<sup>T</sup> and WSH3<sup>T</sup> do not require added Mg<sup>2+</sup> for growth, while WSA2<sup>T</sup> required addition of 1 mM (Table 1).

The pH range for growth was assessed from pH 5.0 to 9.0 at 0.5 unit intervals. pH was adjusted using the following buffers at 50 mM: MES (pH 5.0, 6.0, 6.5), HEPES (pH 7.0, 7.5, 8.0), MOPS (pH 7.0), Tricine (pH 7.5, 8.0), TAPS (pH 8.0) and CHES (pH 8.5, 9.0). The magnesium concentration in the test media was reduced to 10 mM, and media were filter sterilized to avoid precipitation. Following incubation at 40 °C for

PCN9<sup>T</sup> and WSA2<sup>T</sup> or 50 °C for WSH3<sup>T</sup> the pH of all buffered media shifted so that final pH was closer to the optima (Table S1). pH optima and growth ranges were determined based on initial pH of the media. PCN9<sup>T</sup> and WSH3<sup>T</sup> grew optimally between pH 7 and 8, with a range of pH 6.5–8.5. WSA2<sup>T</sup> grew optimally between pH 6.5 and 8.0, with a range of pH 6.0–8.5 (Table 1). All grew aerobically or weakly under anoxic conditions with nitrate and some cells exhibited motility in early exponential phase under oxic conditions. PCN9<sup>T</sup> and WSA2<sup>T</sup> were also capable of reducing perchlorate to chlorite under anoxic conditions as well as oxidizing carbon monoxide as described by Myers King and [8]. Strain WSH3<sup>T</sup> was also shown to be capable of carbon monoxide oxidation under aerobic conditions (Fig. S3).

Substrate utilization was assessed with single carbon sources at a final concentration of 25 mM in 3.8 M NaCl CM1 medium. Cells used for inoculating media were harvested by centrifugation (10 min, 4 °C, 10000 g), washed twice and re-suspended in substrate-free CM1; growth was monitored spectrophotometrically (OD<sub>600</sub>) for 14 days. Substrates included malic acid, glycine, sodium glycolate, glucuronic acid, gluconic acid, pyruvic acid, succinic acid, proline, mannitol, alanine, malonic acid, sodium citrate, aspartic acid, valine, serine, sodium acetate, propionic acid, sodium tartrate, glycine betaine, sodium fumarate, trimethylamine, dimethylamine, methylamine, sodium lactate, glycerol, acetone, ethanol, isopropanol, methanol, glucose, galactose, lactose, sucrose, ribose, mannose, arabinose, xylose, fructose, peptone, casamino acids and tryptone. PCN9<sup>T</sup> grew with the following sole carbon sources: pyruvic acid, proline, alanine, sodium acetate, glycerol, glucose, mannose and tryptone. WSA2<sup>T</sup> grew with gluconic acid, succinic acid, pyruvic acid, proline, alanine, sodium acetate, sodium fumarate, sodium lactate, glucose, tryptone and peptone. WSH3<sup>T</sup> grew with pyruvic acid, mannitol, sodium acetate and sodium lactate (Table 1).

Catalase activity was determined by combining a colony and hydrogen peroxide and observing formation of oxygen bubbles. Oxidase activity was determined by adding oxidase reagent onto colonies grown on 3.8 M NaCl CM1-pyruvate agar plates. WSA2<sup>T</sup> and WSH3<sup>T</sup> were positive for catalase while PCN9<sup>T</sup> was negative. PCN9<sup>T</sup>, WSA2<sup>T</sup> and WSH3<sup>T</sup> were

**Table 1.** Differential characteristics of strains PCN7<sup>T</sup>, WSA2<sup>T</sup> and WSH3<sup>T</sup> with closest related species identified by 16S rRNA gene similarityStrains: 1, PCN9<sup>T</sup>; 2, *Halobacterium noricense* A1<sup>T</sup>; 3, WSA2<sup>T</sup>; 4, *Halobaculum roseum* D90<sup>T</sup>; 5, WSH3<sup>T</sup>. +, Positive; –, negative; ND, not determined.

Characteristic	1	2	3	4	5
Cell shape	Rods	Rods	Rods	Rods	Rods
Colony colour	Pink	Red	Pink	Pink	Red
Temperature for growth (°C)					
Optimum	40	45	50	40	50
Range	15–55	28–50	25–60	25–50	30–60
pH for growth:					
Optimum	7.0–8.0	5.2–7.0	6.5–8.0	7.0	7.0–8.0
Range	6.5–8.5	5.1–8.8	6.0–8.5	6.5–8.0	6.5–8.5
NaCl (M) for growth:					
Optimum	3	3	3	3.4	4
Range	2.0–5.4	2.1–ND	2.0–5.4	1.7–5.1	3.0–5.4
Catalase activity	–	+	+	+	+
Oxidase activity	+	–	+	–	+
Denitrification	–	–	–	–	–
Nitrate reduction to nitrite	+	–	+	+	–
CO oxidation	+	–	+	–	+
DNA G+C content (mol%)	66.75	54.3–54.5	67.62	65.9	63.97
Hydrolysis of:					
Tween 20	–	–	–	–	+
Tween 80	–	–	–	–	+
Gelatin	+	–	+	–	–
Starch	–	–	+	–	–
Growth substrates:					
Gluconic acid	–	+	+	–	–
Pyruvic acid	+	+	+	+	+
Succinic acid	–	+	+	+	–
Proline	+	–	+	–	–
Mannitol	–	–	–	+	+
Alanine	+	–	+	–	–
Sodium citrate	–	+	–	–	–
Sodium acetate	+	+	+	–	+
Sodium fumarate	–	+	+	+	–
Sodium lactate	–	–	+	–	+
Glycerol	+	+	–	+	–
Glucose	+	–	+	+	–
Galactose	–	–	–	+	–
Lactose	–	–	–	–	–

Continued

Table 1. Continued

Characteristic	1	2	3	4	5
Sucrose	–	–	–	+	–
Ribose	–	–	–	–	–
Mannose	+	–	–	+	–
Xylose	–	–	–	–	–
Fructose	–	+	–	+	–
Tryptone	–	+	+	+	–
Peptone	–	+	+	+	–
Casamino acids	–	+	–	–	–

positive for oxidase activity. PCN9<sup>T</sup> produced indole from tryptophan, while WSA2<sup>T</sup> and WSH3<sup>T</sup> did not. Hydrolysis of starch (0.2%), gelatin (1%), casein (0.1%), Tween 80 (0.1%) and Tween 20 (0.1%) were determined by supplementing plated CM1 media. PCN9<sup>T</sup> could hydrolyse gelatin. WSA2<sup>T</sup> could hydrolyse starch and gelatin. WSH3<sup>T</sup> could hydrolyse Tween 20 and Tween 80. None of the isolates hydrolysed casein, or grew under anoxic conditions with arginine as an electron acceptor (Table 1).

Polar lipids for all strains were determined commercially by the Identification Service of the DSMZ (Braunschweig, Germany). All assays followed standard methods [33–35]. Major polar lipids of PCN9<sup>T</sup> included glycolipid, phospholipid, phosphatidylglycerol and phosphatidylglycerol phosphate methylester. Major polar lipids of WSA2<sup>T</sup> included glycolipid, phospholipid, phosphatidylglycerol, phosphatidylglycerol sulfate, and phosphatidylglycerol phosphate methylester. Major polar lipids of WSH3<sup>T</sup> included phosphatidylglycerol, phosphatidylglycerol sulfate and phosphatidylglycerol phosphate methylester. Differences when compared to their closest relatives include: the additional presence of phosphatidylglycerol sulfate, triglycosyl diether and sulfated tetraglycosyl diether in *Halobacterium noricense*, and the absence of phosphatidylglycerol sulfate in both *Halobaculum roseum* and *Halovenus aranensis*.

The DNA base composition (G+C) for all strains was calculated from genome sequence data. Values of 66.75, 67.62 and 63.97 mol% were obtained for PCN9<sup>T</sup>, WSA2<sup>T</sup> and WSH3<sup>T</sup>, respectively (Table 1).

## DESCRIPTION OF *HALOBACTERIUM BONNEVILLEI* SP. NOV.

*Halobacterium bonnevilliei* (*bon.ne.vil'le.i* N.L. gen. n. *bonnevilliei* of Bonneville, honours Major Benjamin Bonneville, for whom Bonneville Salt Flats is named).

Cells are Gram-stain-negative, sometimes motile rods of varying length. When grown on solid media colonies are round, smooth, convex, shiny, with opaque pink pigmentation. Growth occurs at 2.0–5.4 M NaCl (optimum, 3 M), at

15–55 °C (optimum, 40 °C) and at pH 6.5–8.5 (optimum, pH 7–8). Added magnesium is not required. Cells can reduce nitrate to nitrite and reduce perchlorate to chlorite. Cells are positive for gelatin hydrolysis and oxidase activity. Capable of CO oxidation; possesses a form I large sub-unit CO dehydrogenase gene (*coxL*) and a canonical form I *cox* operon. Utilizes the following substrates as sole carbon sources for growth: pyruvic acid, proline, alanine, sodium acetate, glycerol, glucose and mannose. Substrates not utilized for growth include: malic acid, glycine, sodium glycorate, glucuronic acid, gluconic acid, succinic acid, mannitol, malonic acid, sodium citrate, aspartic acid, valine, serine, propionic acid, sodium tartrate, sodium lactate, glycine betaine, sodium fumarate, trimethylamine, dimethylamine, methylamine, acetone, ethanol, isopropanol, methanol, sucrose, fructose, galactose, lactose, ribose, arabinose, xylose, peptone and casamino acids. The major polar lipids include: glycolipid, phospholipid, phosphatidylglycerol and phosphatidylglycerol phosphate methylester. The DNA G+C content is 66.75 mol%. The type strain is PCN9<sup>T</sup> (=JCM 323472=LMG 31022=ATCC TSD-126) isolated from a salt crust sample of the Bonneville Salt Flats (UT, USA).

## DESCRIPTION OF *HALOBACULUM SALITERRAE* SP. NOV.

*Halobaculum saliterrae* (*sa.li.ter'rae*. L. masc. n. *sal* salt; L. fem. n. *terrae* land; N.L. gen. n. *saliterrae* of saline soil).

Cells are Gram-negative, non-motile, pleomorphic rods. When grown on solid media, colonies are round, smooth, convex, shiny, with opaque pink pigmentation. Growth occurs at 2.0–5.4 M NaCl (optimum, 3 M), at 25–60 °C (optimum, 50 °C) and at pH 6.08.5 (optimum, pH 6.5–8.0). Cells require a minimum addition of 1 mM Mg<sup>2+</sup>, and can reduce nitrate to nitrite. Cells are weakly positive for catalase and oxidase. Capable of starch and gelatin hydrolysis. Cells are able to reduce nitrate to nitrite via dissimilatory nitrate reductase, as well as reduce perchlorate to chlorite. Capable of carbon monoxide oxidation and possesses a form I large sub-unit CO dehydrogenase gene (*coxL*) and a canonical form I *cox*

operon. Can utilize the following substrates as carbon sources for growth: gluconic acid, succinic acid, pyruvic acid, proline, alanine, sodium acetate, sodium fumarate, sodium lactate, glucose, tryptone and peptone. Substrates not utilized for growth include: malic acid, glycine, sodium glycolate, glucuronic acid, mannitol, malonic acid, sodium citrate, aspartic acid, valine, serine, propionic acid, sodium tartrate, glycine betaine, trimethylamine, dimethylamine, methylamine, glycerol, acetone, ethanol, isopropanol, methanol, galactose, lactose, sucrose, ribose, mannose, arabinose, xylose, fructose and casamino acids. The major polar lipids include: glycolipid, phospholipid, phosphatidylglycerol, phosphatidylglycerol sulfate and phosphatidylglycerol phosphate methylester. The DNA G+C content is 67.62 mol%. The type strain is WSA2<sup>T</sup> (=JCM 32473<sup>T</sup>=ATCC TSD-127<sup>T</sup>), isolated from saline soil sampled south of Wendover, Utah, USA.

## DESCRIPTION OF *HALOVENUS CARBOXIDIVORANS* SP. NOV.

*Halovenus carboxidivorans* (*car.bo.xi.di.vo'rans*. N.L. neut n. *carboxidum* carbon monoxide; L. pres. part. adj. *vorans* devouring; N.L. part. adj. *carboxidivorans* carbon monoxide-devouring).

Cells are Gram-negative, non-motile, pleomorphic rods. When grown on solid media, colonies are round, smooth, convex, shiny, with opaque red pigmentation. Growth occurs at 3.0–5.4 M NaCl (optimum, 4 M), at 30–60°C (optimum, 50°C) and at pH 6.5–8.5 (optimum, pH 7–8). Added magnesium is not required for growth. Cells are positive for catalase and oxidase. Nitrate is reduced to nitrite. Hydrolysis of Tween 20 and Tween 80. Capable of CO oxidation, and possesses a form I large sub-unit CO dehydrogenase gene (*coxL*) and a canonical form I *cox* operon. Can utilize the following substrates as carbon sources for growth: pyruvic acid, mannitol, sodium acetate and sodium lactate. Substrates not utilized for growth include: gluconic acid, succinic acid, proline, alanine, sodium fumarate, glucose, tryptone, peptone, malic acid, glycine, sodium glycolate, glucuronic acid, malonic acid, sodium citrate, aspartic acid, valine, serine, propionic acid, sodium tartrate, glycine betaine, trimethylamine, dimethylamine, methylamine, glycerol, acetone, ethanol, isopropanol, methanol, galactose, lactose, sucrose, ribose, mannose, arabinose, xylose, fructose and casamino acids. The major polar lipids include: phosphatidylglycerol, phosphatidylglycerol sulfate and phosphatidylglycerol phosphate methylester. The DNA G+C content is 63.97 mol%. The type strain is WSH3<sup>T</sup> (=JCM 32474<sup>T</sup>=ATCC TSD-128<sup>T</sup>), isolated from saline soil sampled south of Wendover, Utah, USA.

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### Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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